Genomics and Proteomics: Diagnosis and Treatment Applications in Brain Trauma

Jitendra R. Dave
Department of Neuropharmacology and Molecular Biology
Division of Neurosciences
WRAIR, Silver Spring, MD-20910

Genomics:

In the past several years, our studies have documented changes in targeted or selected genes following brain injury using QRT-PCR. Although this approach is critical and hypothesis driven, it does not allow broad and detailed assessments of changes in the expression of various gene families following insults to the brain.

Recent developments in genomics with the availability of smaller and specific functional DNA microarray chips inspired us to initiate studies in this research arena.

QRT/PCR versus Microarray

QRT/PCR: Precise and accurate measure of mRNA of a single gene at a time. Not suitable for rapid and quick screening of large number of gene families.

Microarray: Suitable for rapid and quick screening of large number of gene families. Yield only qualitative changes of gene expression and is not always accurate. Needs additional confirmation by QRT/PCR

Affymetrix® Rat Neurobiology Chip

(2500 gene probes with 1320 functional gene probes)

GeneChip® Probe Array

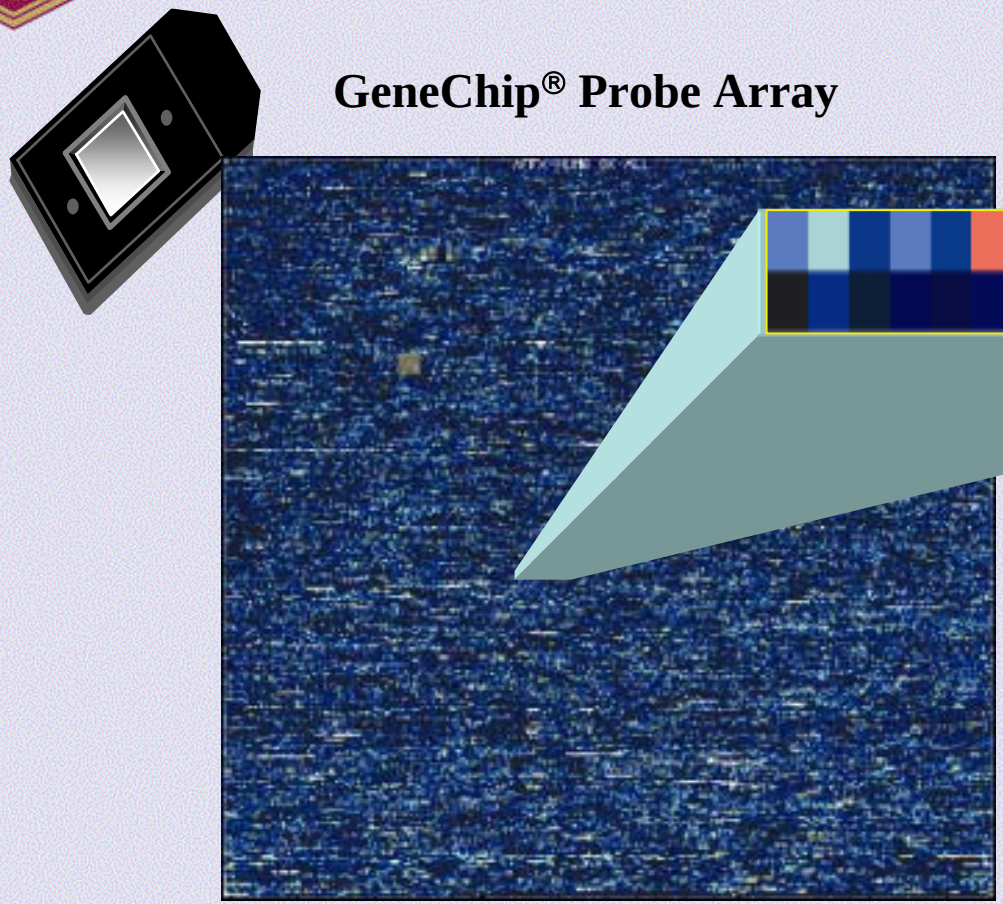


Image of Hybridized Probe Array

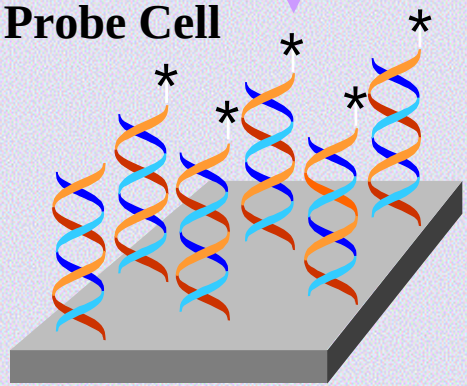
Probe Pair

Probe Set

PM

MM

Hybridized Probe Cell

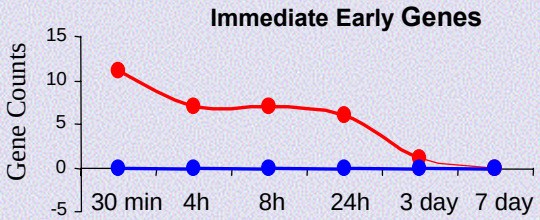


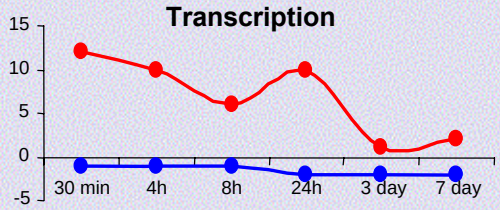
Probe Cell
(feature)

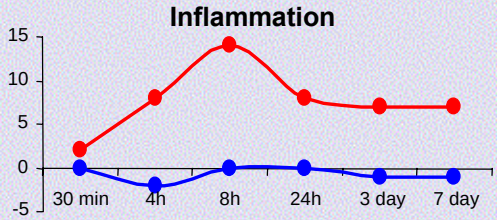
Gene Function	Up	Down
Inflammation	20	3
Immediately early gene	17	0
Apoptosis	17	1
Growth Factor	17	2
Structure	17	6
Transcription	15	3
Metabolism	13	7
Heat Shock Protein	8	1
Signal transduction	19	20
Ion channels	14	22
Neurotransmission/release	7	26
Miscellaneous	14	15
Total (284)	178	106

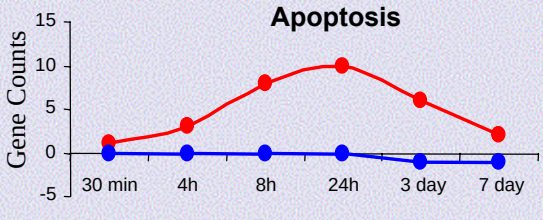
(Following Brain injury)

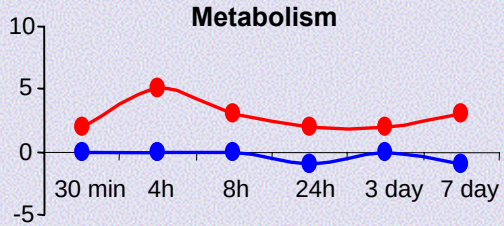
Temporal Profile of Genomic Response after Ischemic Brain Injury

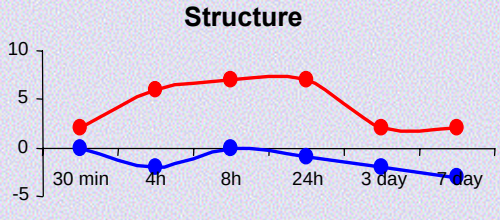


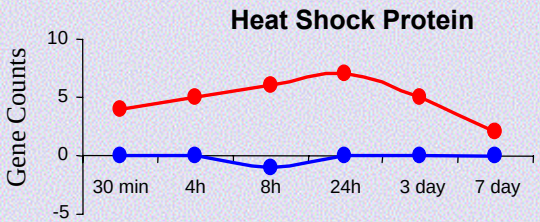


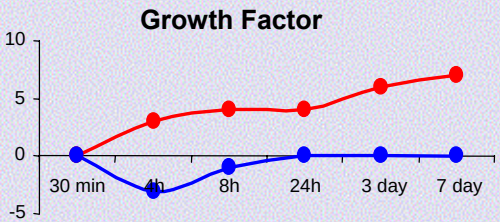


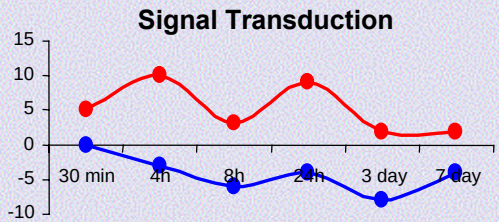


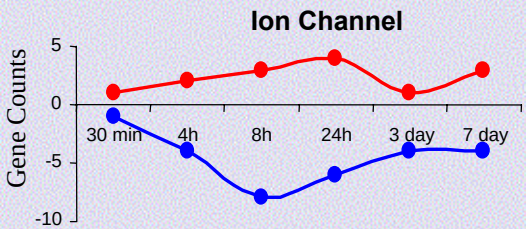


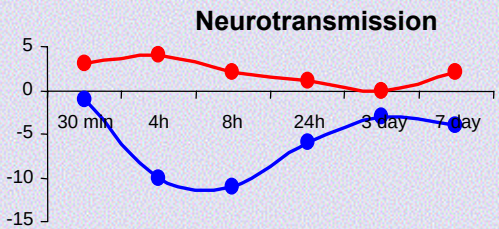












Up-Regulated Gene Counts

Down-Regulated Gene Counts

Apoptosis

Gene Name	30 min	4 h	8 h	24 h	3 day	7 day
Bcl-xβ	3.4	4.5	5.6	3.8		
DP5		2.8	4.0			
Fas antigen ligand		4.1	4.4	6.1		
inhibitor of apoptosis p			2.6	3.0		
Bcl-2			3.0			
calpain large subunit (nCL-4)			4.6	3.9		
casepase 2			5.2			
caspase-7			3.2	5.5		
p94 (calpain 3) p				2.6		
IL-1β CE related p				3.1		
IL-1β CE				2.6	3.8	
rBax α				3.8	3.0	
IGIF isoform α prec.					6.3	
SP120,					2.3	
Nedd2/Ich-1					5.6	7.9
nuclear oncoprotein p53					10.9	5.4
calpain isoform Lp85						3.9

Proteomics:

A powerful research tool that extends our molecular evaluations beyond ‘genomics’ in that it can not only identify up- or down-regulation of functional proteins, but can also identify specific protein modifications (e.g. phosphorylation, methylation, truncation etc) following an insult.

Ultimate goals of our studies:

- **Identify protein changes specific to the brain injury, and correlate these changes in CSF & plasma for development of possible biomarkers.**
- **Use the proteomics information to develop a "diagnostic biomarker capability" to identify not only the severity but also anatomical extent of injury.**
- **Develop therapeutic interventions based on changes in specific protein families.**

Although these are very ambitious goals, but are achievable by extensive studies.

Why study the Proteome?

Genome: The blue-print plan for Man

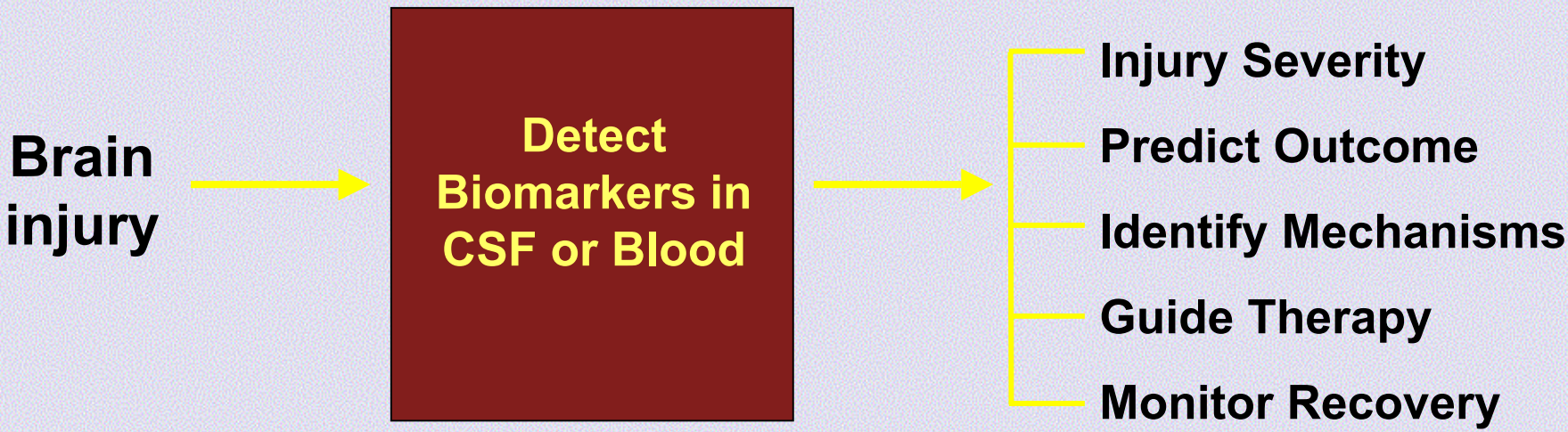
Proteome: The parts-list for Man

All of the cell's working parts are proteins

Bio-Markers of Brain Injury

- **Biomarkers**
  - measurable internal indicators of changes in organisms at the molecular or cellular level

Develop biomarkers that can be measured in blood or CSF



Classical Proteomics

Separation of proteins →
2D gel electrophoresis

Characterization of proteins →
Mass spectrometry

Similar to our collaborative efforts with Dr. Maryanne Vahey (WRAIR-Microarray core facility, Rockville), we have established a proteomics collaboration with Dr. Ronald Hayes (McKnight Foundation, University of Florida) and initiated a comprehensive, time-dependent analysis of proteomic changes in brain, CSF and plasma following ischemic and traumatic experimental brain injury.

Participants

WRAIR-Silver Spring

Dr. F.C. Tortella
Dr. X-C, M. Lu
Dr. C. Yao
Dr. R. Berti
Dr. A. Williams
Dr. R.W. Chen

WRAIR-Rockville

Dr. M. Vahey

Univ. Of Florida

Dr. R. Hayes
Dr. B. Pike
Dr. K. Wang